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Delivery Systems in Wound Healing and Nanomedicine

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Abstract

Introduction: Delivery systems in nanomedicine contribute to the improvements in wound healing, tissue regeneration, and anticancer pharmacological fields. Although various wound dressings have been used in wound care treatments, there is a great challenge in the wound management of ulcers, trauma, chronic wounds, and severe injury and burns, especially infected wounds.

Body: To accelerate wound healing, influence tissue repair, reduce scarring, and control infection, various delivery devices have been developed in wound healing. The application of delivery devices has improved early as well as long-term wound care in delayed healing wounds. Main delivery systems are described, including drugs, bioactive proteins/growth factors, genes, and cells, outlining the advantages and limitations of each carrier in wound healing, as well as the mechanisms and release. This chapter reviews biomaterials and scaffolds that provide the carriers of bioactive agents, which include antimicrobial agents, combinations of cells, growth factors and genes, both scaffolds and cell interactions toward regeneration of skin tissues, vascular reconstructions, as well as transdermal carriers. In addition, the regulations, procedures, and clinical trails for delivery systems for wound healing are discussed.

Conclusion: In the past decades, many wound dressings and skin substitutes have been developed to treat skin loss and wounds. Delivery systems can improve wound healing and tissue regeneration. Looking toward the future, the need for delivery wound healing products for chronic and complex wounds will increase. Functionalized delivery systems will probably be the academic interest and industrial focus on wound healing.

Keywords: wound healing, delivery system, wound dressing, skin regeneration, bio-materials

1. Introduction

Nanomedicine has had a significant impact on delivery system development for pharmacological fields that include controlled-release wound dressings and biocompatible nanocarriers for biomedical applications [1]. As the largest organ in the human body, skin gives the body protection, but in so doing sustains a variety of skin wounds that require immediate repair process [2]. Modern wound dressings have been under development for decades. Although there are a wide array of wound dressings, ointments, and medical devices for clinical use, the time-consuming process of wound management is mainly restricted to wound repair rather than regeneration, which are two distinct definitions [3]. The key problem of skin regeneration is how to restore the native structure and function of the injured organ, including blood capillaries. Recently, biomaterial carriers in nanomedicine have shifted the focus from patient survival to quality of skin regeneration in terms of function, scar reduction, and improved aesthetics for reconstruction surgeries and burns [4]. In the formats of wound dressings and transdermal formulations, delivery systems have been applied to accelerate wound healing and to promote tissue regeneration, as well as to treat skin cancers using nanomedicine.

There are different circumstances in which people may need wound care and management. To meet the challenges of wound treatments for acute wounds and chronic wounds, such as large-area skin loss, burns, ulcers (pressure, diabetic, neuropathic, or ischemic), trauma, and especially infected wounds, which are mostly caused by microbes [5], the accurate delivery of antimicrobial agents is attracting much attention from researchers [6–8]. In addition to antimicrobial wound dressing, delivery systems of bioactive proteins, such as peptides and growth factors (platelet-derived growth factor, PDGF; endothelial growth factor, EGF; and fibroblast growth factor 2, FGF2 or bFGF), have demonstrated their promising effects in wound healing [9]. Cell therapy, including stem cell strategy, provides a novel therapeutic approach to wound healing [10]. Interestingly, mesenchymal stem cells (MSCs) and adipose-derived stem cells (ASCs) have emerged as a new approach in skin tissue engineering to accelerate wound closure, which would be of enormous benefit particularly for those wounds experiencing delayed healing in patients with diabetes and elderly [11, 12]. Gene delivery systems for wound healing have been also developed to transfer deoxyribonucleic acid (DNA) and ribonucleic acid (RNA) to wound sites [13, 14]. The regulations of delivery systems in wound healing can be complicated and vary greatly depending on the specific biomaterials and scaffolds, as well as the clinical use in particular [15]. In the commercialization of delivery wound healing systems, developmental and regulatory challenges are greater than in normal wound dressing and wound healing products. The biomaterials and scaffolds used in delivery systems take advantage of different structures, chemical parameters, and sources and so may require more rigorous development and regulation.

This chapter reviews biomaterials and scaffolds used in the design, characterization, and evaluation of delivery systems for wound healing, which include delivering antimicrobial drugs, combinations of proteins (growth factors and peptides), cells, and genes (**Figure 1**). Specific examples of application are summarized. Regenerations of skin tissues and recon-

structions of blood capillaries in the wound care process are covered. In addition, the regulatory considerations for delivery systems in the wound healing field are also explored.

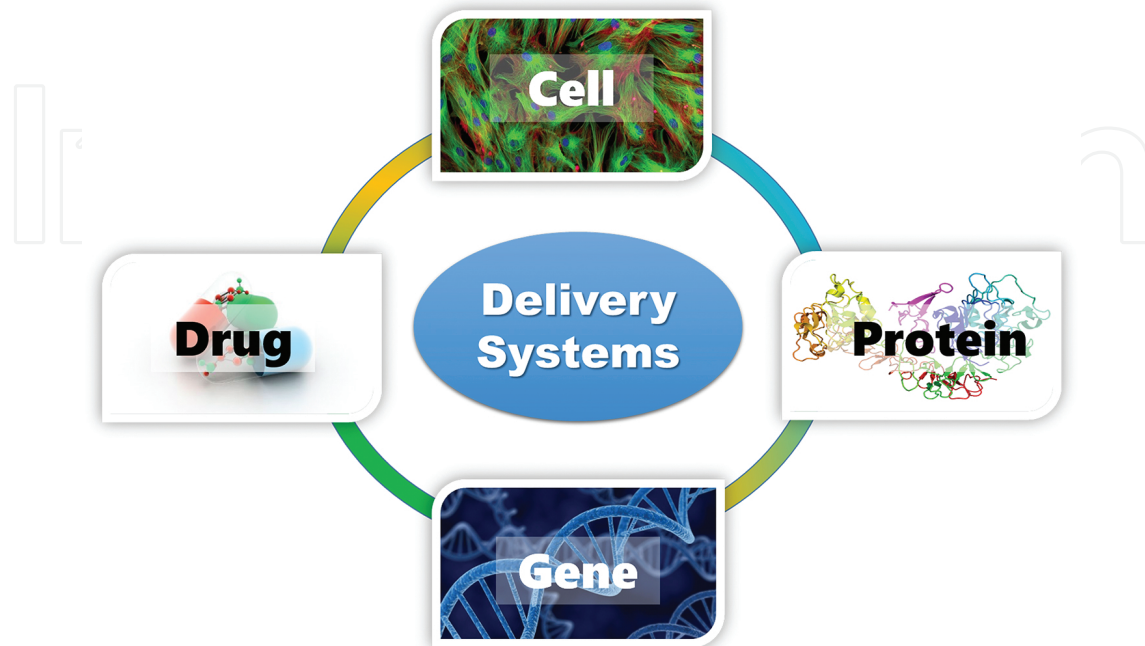


Figure 1. Delivery systems in wound healing.

2. Drug delivery system in wound healing

Chronic wounds and infected wounds currently pose a significant burden worldwide. Drug delivery systems (DDS) in wound healing that release antimicrobial and anti-inflammatory drugs represent a great opportunity to prevent infections or enhance the effectiveness of current commercial drugs. Many biocompatible biomaterials have been extensively investigated to deliver drugs into wound beds and to improve wound healing. Significant efforts have been made to develop DDS using different types of biomaterials, such as polymeric microspheres and nanospheres, lipid nanoparticles, nanofibrous structures, hydrogels, and scaffolds [16].

2.1. Delivery of antibiotics

Wound healing is a complex process that often requires treatment with antibiotics. To optimize and improve the usage of currently available antibiotics, DDS of antibiotics have attracted much attention. Antibiotic drugs used in delivery systems for wound healing are cefazolin [17], gentamicin sulfate [6], ceftazidime pentahydrate [18], ciprofloxacin [19], gentamicin [20], doxycycline hyclate [21], and the anti-inflammatory drug diclofenac [20]. Various biodegradable polymeric scaffolds (electrospun nanofibers, microspheres, composites, and films) were

investigated for antibiotic delivery systems, including electrospun nanofibers of poly(lactide-co-glycolide) (PLAGA) [17], composites of a polyglyconate core and a porous poly(DL-lactic-co-glycolic acid) shell [18], chitosan (CS)-gelatin composite films [19], a three-dimensional (3D) polycaprolactone-tricalcium phosphate (PCL-TCP) mesh [6], bacterial cellulose (BC) membranes grafted with RGDC peptides (R for arginine, G for glycine, D for aspartic acid, C for cysteine) [20], poly(vinyl alcohol) (PVA) microspheres sandwiched poly(3-hydroxybutyric acid) (PHB) electrospun fibers [21], and β -cyclodextrin-conjugated hyaluronan hydrogels [22].

Antibiotic agents used in wound healing typically incur adverse effects (e.g., nephrotoxicity for vancomycin, cytotoxicity for ciprofloxacin, and hemolysis for antimicrobial polymers). Loading of antibiotics within polymeric vesicles could attenuate side effects, which has been demonstrated recently [23]. Li et al. reported a general strategy to construct a bacterial strain-selective delivery system for antibiotics based on responsive polymeric vesicles. That was in response to enzymes, including penicillin G amidase (PGA) and β -lactamase (Bla) that are closely associated with drug-resistant bacterial strains. A sustained release of antibiotics enhanced stability and reduced side effects. The results demonstrated that methicillin-resistant *Staphylococcus aureus* (*S. aureus*) (MRSA)-triggered release of antibiotics from Bla-degradable polymeric vesicles *in vitro* inhibited MRSA growth, and enhanced wound healing in an *in vivo* murine model.

2.2. Delivery of silver

To solve the problem of the increased prevalence and growth of multidrug-resistant bacteria, silver is used to reduce and eliminate wound infections using methodologies that limit the ability of bacteria to evolve into further antibiotic-resistant strains. In recent decades, the developments of silver (colloidal silver solution, silver proteins, silver salts, silver sulfadiazine (SSD) and nanosilver)-containing wound dressings for healing promotion and infection reduction have provided promising approaches [24]. The main synthesis approaches of silver monocrystalline silver (nanosilver or silver nanoparticle) include chemical reduction, micro-organism reduction, microwave-assisted photochemical reduction, and laser ablation. Antibacterial wound dressings in the formats of AgNP-embedded poly(vinyl pyrrolidone) (PVP) hydrogels were prepared by γ -irradiation at various doses: 25, 35, and 45 kGy [25]. Antibacterial tests showed that the 1 and 5 mM AgNP-embedded PVP hydrogels were effective, with 99.99% bactericidal activity at 12 and 6 h, respectively. A gamma-irradiated PVA/nanosilver hydrogel was also developed for potential use in burn dressing applications [26]. Interestingly, the wound healing activity of 0.1% w/w AgNPs in Pluronic F127 gels was enhanced to a considerable extent [27]. A new type of high surface area metallic silver in the form of highly porous silver microparticles (AgMPs) was studied [28]. Polylactic acid (PLA) nanofibers were successfully loaded with either highly porous AgMPs or AgNPs. A simulated three-dimensional (3D) coculture system was designed to evaluate human epidermal keratinocytes and *S. aureus* bacteria on the wound dressings. PLA nanofibers containing highly porous AgMPs exhibited steady silver ion release at a greater rate of release than nanofibers containing AgNPs.

Due to its antimicrobial activity, good coagulation and immunostimulating activities, chitosan is one of the native polymers chosen to control infection and enhance wound healing. Chitosan-based wound dressings can be gels, microparticles or nanoparticles, sponges and films [29]. Sacco et al. combined the two antimicrobial agents, silver and chitosan, to develop a silver-containing antimicrobial membrane based on chitosan-tripolyphosphate (TPP) hydrogel for wound treatments. Based on the slow diffusion of TPP, the macroscopic chitosan hydrogels were obtained that included AgNPs stabilized by a lactose-modified chitosan. Besides the good bactericidal properties of the material, the biocompatibility assays on keratinocytes (HaCaT) and fibroblasts (NIH-3T3) cell lines did not prove to have any harmful effects on the viability of cells using the MTT [1-(4,5-dimethylthiazol-2-yl)-3,5-diphenylformazan] method [8]. Chitin was also used to form the composite scaffolds with nanosilver. These chitin/nanosilver composites were found to be bactericidal against *S. aureus* and *Escherichia coli* (*E. coli*) with good blood-clotting ability [30].

Bioelectric wound dressing can also deliver silver to wound beds. *Pseudomonas aeruginosa* (*P. aeruginosa*) is a common bacterium associated with chronic wound infection. An US Food and Drug Administration (FDA)-approved wireless electroceutical dressing (WED), which in the presence of conductive wound exudate is activated to generate an electric field (0.3–0.9 V), was investigated to test its anti-biofilm properties using a pathogenic *P. aeruginosa* strain PAO1. WED markedly disrupted biofilm integrity in a setting where normal silver dressing was ineffective. Biofilm thickness and number of live bacterial cells were decreased in the presence of WED because WED served a spontaneous source of reactive oxygen species [31].

2.3. Delivery of other drugs

Besides silver, other drugs can be used to improve wound healing, for example, the anti-scar drug astragaloside IV [32]. In a rat full-skin excision model, the**** *in vivo* regulation of 9% astragaloside IV-based solid lipid nanoparticles-gel enhanced the migration and proliferation of keratinocytes, increased drug uptake on fibroblasts *in vitro* ($P < 0.01$) through the caveolae endocytosis pathway, and inhibited scar formation *in vivo* by increasing wound closure rate ($P < 0.05$) and by contributing to angiogenesis and collagen regular organization.

Different from most antibiotics that select for resistant bacteria, curcumin acts using multiple mechanisms. Curcumin (diferuloylmethane) is a bioactive and major phenolic component of turmeric derived from the rhizomes of *Curcuma longa* linn. Owing to its antioxidant and anti-inflammatory properties, curcumin plays a significant beneficial and pleiotropic regulatory role not only in cancers, cardiovascular disease, Alzheimer's disease, inflammatory disorders, and neurological disorders but also in wound healing because of its innate antimicrobial properties. However, the clinical implication of native curcumin is hindered due to low solubility, physicochemical instability, poor bioavailability, rapid metabolism, and poor pharmacokinetics, but these issues can be overcome by efficient delivery systems [33]. A biodegradable sponge, made from chitosan (CS) and sodium alginate (SA) with water uptake ability ranging between 1000 and 4300%, was developed to deliver curcumin as a wound dressing material up to 20 days. The *in vivo* animal test using SD rats showed that this CS/SA sponge had a better effect than cotton gauze, and adding curcumin into the sponge enhanced

the therapeutic healing effect and improved collagen arrangement [34]. Curcumin nanoparticles (Curc-np) with an average diameter of 222 ± 14 nm were synthesized [35]. Curc-np represent a significant advance for reducing bacterial load. They can inhibit *in vitro* growth of methicillin-resistant *S. aureus* (MRSA) and *P. aeruginosa* in dose-dependent fashion, and so may represent a novel topical antimicrobial and wound healing adjuvant for infected burn wounds and other cutaneous injuries. Bacterial cellulose (BC) can be used for drug loading and controlled release [36]. The topical or transdermal drug delivery systems of two model drugs (lidocaine hydrochloride and ibuprofen) were developed. Diffusion studies with Franz cells showed that the incorporation of lidocaine hydrochloride in BC membranes provided lower permeation rates than those obtained with the conventional formulations [37].

There is a high mortality in patients with diabetes and severe pressure ulcers, resulting from the reduced neovascularization caused by the impaired activity of the transcription factor hypoxia-inducible factor-1 alpha (HIF-1 α). To improve HIF-1 α activity, Duscher et al. developed the drug delivery system of an FDA-approved small molecule deferoxamine (DFO), which is an iron chelator that increases HIF-1 α transactivation in diabetes by preventing iron-catalyzed reactive oxygen stress [38]. The animal study on a pressure-induced ulcer model in diabetic mice showed a significantly improved wound healing using the transdermal delivery of DFO. DFO-treated wounds demonstrated increased collagen density, improved neovascularization, and reduction of free radical formation, leading to decreased cell death.

3. Bioactive protein delivery systems in wound healing

Wound healing in skin is an evolutionarily conserved, complex, multicellular process, which is executed and regulated by an equally complex signaling network involving numerous growth factors, cytokines, and chemokines [39]. Growth factors are soluble secreted proteins capable of affecting a variety of cellular processes important for tissue regeneration. However, the application of growth factors in clinics remains limited due to lack of good delivery systems and carriers. Recently, biomaterial carriers and sophisticated delivery systems such as nanoparticles and nanofibers for delivery of growth factors and peptides related in wound healing are a main focus in this research area [40].

3.1. Delivery of growth factors

EGF, PDGF, FGF2, keratinocyte growth factor (KGF) [41], transforming growth factor- β (TGF- β), insulin-like growth factor (IGF), vascular endothelial growth factor (VEGF), granulocyte macrophage colony stimulating factor (GM-CSF), and connective tissue growth factor (CTGF) are the main growth factors correlated with the wound healing process of skin [16]. Growth factors usually have short half-life time leading to a rapid deactivation at local wound beds in the body and resulting in a low efficacy. In order to enhance the efficacy of growth factor delivery systems, some bioactive and biodegradable matrixes including extracellular matrixes, have been used as carriers [42].

EGF is one of the most common growth factors used for treating skin wounds. Succinoylated dextrin (~85,000 g/mol; ~19 mol% succinoylation), a clinically well-tolerated polymer, was used to deliver EGF and led to sustained release of free recombinant human EGF over time (52.7% release after 168 h) [43]. Using a layer-by-layer assembly technique, EGF was successfully encapsulated using poly(acrylic acid) (PAA)-modified polyurethane (PU) films [44] or chitosan and alginate films [45]. Johnson and Wang treated the full-thickness wounded mice with a heparin-binding epidermal growth factor coacervate delivery system, and the results exhibited the enhanced migration of keratinocytes with retained proliferative potential, forming a confluent layer for regained barrier function within 7 days [46]. Chitosan-based gel formulations containing egg yolk oil and EGF are better alternatives compared to Silverdin® (1% silver sulfadiazine), given their significant difference ($P < 0.05$) treating wounds in Wistar rats [47]. Since the healing rate of wound is an important factor influencing the outcome of clinical treatments, as well as a crucial step in burn wound treatment, and the quality of wound healing has a direct bearing on the life quality of patients, FGF2 clearly has clinical efficacy in a variety of wound managements [48]. Skin flap survival is a major challenge in reconstructive plastic surgery. A sustained delivery system of FGF2 using heparin-conjugated fibrin was used to improve skin flap survival significantly in a rat animal model [49]. A delivery system composed of fibrin hydrogels doped with bFGF-loaded double emulsion increased the proliferation of endothelial cells compared to sham controls, indicating that the released bFGF was bioactive [50]. An injectable delivery system of PDGF using two-component polyurethane scaffolds was reported to achieve a sustained release for up to 21 days. The *in vitro* bioactivity of the released PDGF was largely preserved by a lyophilized powder. The presence of PDGF attracted both fibroblasts and mononuclear cells, significantly accelerating the degradation of the polymer and enhancing the formation of new granulation tissue as early as day 3 [51]. Hyaluronan-based porous nanoparticles were also investigated for the delivery of PDGF [52]. Recombinant human stromal cell-derived factor-1 (SDF-1), a naturally occurring chemokine that is rapidly overexpressed in response to tissue injury, was delivered in an alginate gel to accelerate wound closure and reduce scar formation [53]. SDF-1 delivery systems were evaluated using an acute surgical Yorkshire pig model. Wounds treated with SDF-1 protein ($n = 10$) and plasmid ($n = 6$)-loaded alginate patches healed faster than the sham ($n = 4$) or control ($n = 4$). At day 9, SDF-1-treated wounds significantly accelerated wound closure ($55.0 \pm 14.3\%$ healed) compared to nontreated controls ($8.2 \pm 6.0\%$, $p < 0.05$).

Recently, it has been increasingly recognized that biodegradable and biocompatible scaffolds incorporated with multiple growth factors might serve as the most promising medical devices for skin tissue regeneration. Beyond drug delivery, BC hydrogel is used to deliver bFGF, EGF, and KGF with modifications of different extracellular matrices (ECMs; collagen, elastin, and hyaluronan) [54]. *In vitro* and *in vivo* evaluation of the applicability of a dextran hydrogel loaded with chitosan microparticles ($255 \pm 0.9 \mu\text{m}$) containing EGF and VEGF were performed, and they accelerated wound healing [55]. Moreover, the histological analysis revealed the absence of reactive or granulomatous inflammatory reaction in skin lesions. Multiple epidermal induction factors (EIF), such as EGF, insulin, hydrocortisone, and retinoic acid (RA), were prepared for blended and core-shell electrospinnings with gelatin (gel) and poly(L-lactic

acid)-co-poly-(ε-caprolactone) (PLLCL) solutions [56]. An initial 44.9% burst release from EIF blended electrospun nanofibers was observed over a period of 15 days. The epidermal differentiation potential of adipose-derived stem cells (ADSCs) was used to evaluate the scaffolds prepared either by core-shell spinning or by blend spinning. After 15 days of cell culture, the proliferation of ADSCs on EIF-encapsulated core-shell nanofibers was the highest. Moreover, a higher percentage of ADSCs were differentiated to epidermal lineages on EIF-encapsulated core-shell nanofibers compared to the cell differentiation of EIF-blended nanofibers, and this can be attributed to the sustained release of EIF from the core-shell nanofibers. A method for coating commercially available nylon wound dressings using the layer-by-layer process was utilized to control the release of VEGF165 and PDGF-BB [57]. Animal evaluation was performed using a db/db mouse model of chronic wound healing. This combination delivery system promotes significant increases in the formation of granulation tissue and/or cellular proliferation when compared to dressings utilizing single growth factor therapeutics.

3.2. Delivery of peptides

Current therapeutic regimens of wounded patients are static and mostly rely on matrices, gels, and engineered skin tissue. Accordingly, there is a need to design next-generation grafting materials to enable biotherapeutic spatiotemporal targeting from clinically approved matrices. Peptides are good candidates for controlling wound infections. A drug carrier system was designed for delivering an insect metalloproteinase inhibitor (IMPI) drug to enable treatment of chronic wound infections [58]. Poly(lactic-co-glycolic acid) (PLGA) supplies lactate that accelerates neovascularization and promotes wound healing. Delivery systems of LL37 peptide encapsulated in PLGA nanoparticles (PLGA-LL37 NP) were evaluated in full-thickness excisional wounds. A significantly higher collagen deposition, re-epithelialized and neovascularized composition were found in PLGA-LL37 NP-treated group. *In vitro*, PLGA-LL37 NP induced enhanced cell migration but had no effect on the metabolism and proliferation of keratinocytes. Interestingly, it displayed antimicrobial activity on *E. coli* [59]. CM11 peptide (WKLFKKILKVL-NH₂) (128 mg/L), a short cecropin-melittin hybrid peptide, was delivered by an alginate sulfate-based hydrogel as the antimicrobial wound dressing, and its healing effects were tested on skin infections caused by MRSA (200 μL, 3 × 10⁸ CFU/mL) in a mouse model [60]. During 8-day period, the 2% mupirocin treatment group and hydrogel containing peptide treatment groups showed similar levels of wound healing.

4. Cell delivery systems in wound healing

Wound healing involves the coordinated efforts of several cell types, including keratinocytes, fibroblasts, endothelial cells, macrophages, and platelets. The migration, infiltration, proliferation, and differentiation of these cells will culminate in an inflammatory response, the formation of new tissue and ultimately wound closure [39]. Cell-based therapies for wound repair are limited by inefficient delivery systems that fail to protect cells from acute inflammatory environments [61]. Wound dressing of cells laden in biomaterials on wound surfaces

might not effectively and timely exert functions on deep or chronic wounds, where insufficient blood supply presents. Therefore, cell delivery systems are the main focus in the cell-based therapeutic field. Cell, including stem cells and other cells, delivered wound dressings have recently shown great promise for accelerating wound healing and reducing scar formation.

4.1. Stem cells

Stem cell therapy offers a promising new technique for aiding in wound healing; however, current findings show that stem cells typically die and/or migrate from the wound site, greatly decreasing the efficacy of the treatment. Most stem cells studied in wound healing delivery systems are mesenchymal stem cells (MSCs), endothelial progenitor cells (EPCs), adipose-derived stem cells (ASCs), umbilical cord perivascular cells (UCPCs), and circulating angiogenic cells (CACs). MSCs have been shown to improve tissue regeneration in several preclinical and clinical trials [62]. MSCs from various sources, such as bone marrow and adipose tissue, have been reported in the delivery systems for wound healing [10, 63].

A 3D membrane (FBMSC-CMM) from a freeze-dried bone marrow mesenchymal stem cells-conditioned medium (FBMSC-CM) can hold over 80% of the paracrine factors, which could significantly accelerate wound healing and enhance the neovascularization as well as epithelialization through strengthening the trophic factors in the wound bed [11]. Scaffolds strongly influence key parameters of stem cell delivery, such as seeding efficiency, cellular distribution, attachment, survival, metabolic activity, and paracrine release [64]. Pullulan was used to form a composite with collagen hydrogel for the delivery of MSCs into wounds [65]. Hydrogels induced MSC secretions of angiogenic cytokines and expression of transcription factors associated with the maintenance of pluripotency and self-renewal (Oct4, Sox2, Klf4) when compared to MSCs grown in standard conditions. Engrafted MSCs were found to differentiate into fibroblasts, pericytes, and endothelial cells but did not contribute to the epidermis. Wounds treated with MSC-seeded hydrogels demonstrated significantly enhanced angiogenesis, which was associated with increased levels of VEGF.

There are other kinds of stem cells that have been used in combination with 3D scaffolds as a promising approach in the field of regenerative medicine. For instance, human umbilical cord perivascular cells (HUCPVC) [66], amniotic fluid-derived stem cells (AFSs) [67], EPCs [68], and circulating angiogenic cells (CACs). CACs are known as early EPCs and are isolated from the mononuclear cell fraction of peripheral blood, and provide a potential topical treatment for nonhealing diabetic foot ulcers. A scaffold fabricated from type 1 collagen facilitates topical cell delivery of CACs to a diabetic rabbit ear wound (alloxan-induced ulcer). Increased angiogenesis and increased percentage wound closure were observed with the treatment of collagen and collagen seeded with CSCs [69].

Compared to MSCs and EPCs, adipose-derived mesenchymal stem cells (ASCs) represent an even more appealing source of stem cells because of their abundance and accessibility. ASCs are autologous, non-immunogenic, plentiful, and easily obtained [70]. An acellular dermal matrix (ADM) scaffold made from cadaveric skins of human donors (AlloDerm, LifeCell Corp., Branchburg, NJ, USA) was served as a carrier for the delivery of ASCs [12]. ASCs-ADM grafts secreted various cytokines, including VEGF, HGF, TGF β , and bFGF. Novel technology and

biocompatible biomaterials have been applied for stem cell delivery. A silk fibroin-chitosan (SFCS) scaffold serving as a delivery vehicle for human adipose-derived stem cells (ASCs) was evaluated in a murine soft tissue injury model [71]. Microvessel density at wound bed biopsy sites at 2 weeks postoperative was significantly higher in the ASC-SFCS group vs. SFCS alone (7.5 ± 1.1 vs. 5.1 ± 1.0 blood vessels per high-power field). A newly developed thermoresponsive poly(ethylene) glycol (PEG)-hyaluronic acid (HA) hybrid hydrogel with multiple acrylate functional groups provides an efficient delivery dressing system for human adipose-derived stem cells (hADSCs) [72]. Although cellular proliferation was inhibited, cellular secretion of growth factors, such as VEGF and PDGF production, increased over 7 days, whereas IL-2 and IFN γ release were unaffected. Injectable gelatin microcryogels (GMs) were used to load human ASCs [73]. The results demonstrated the priming effects of GMs on the upregulation of stemness genes and improved secretion of growth factors of hASCs for potential augmented wound healing. In a full-thickness skin wound model in nude mice, multisite injections and dressings of hASC-laden GMs significantly accelerated the healing compared to free stem cell injection.

4.2. Other cells

Endothelial cells (ECs), keratinocytes, and fibroblasts are the most studied cells in terms of accelerated wound healing and improved skin tissue regeneration. A growing number of studies indicate that endothelial cells (ECs) and endothelial progenitor cells (EPCs) may regulate vascular repair in wound healing via paracrine mechanisms [61]. Using dried reagent patches that incorporate dextran (DEX) and a bulk aqueous phase comprising a cell culture medium containing poly(ethylene) glycol (PEG), Bathany et al. made a micro-patterned localized delivery of fluorescent molecules and enzymes for cell detachment [74]. Keratinocytes were delivered to dermal wounds in mice via cell-adhesive peptides attached to chitosan membranes [75]. Two peptides of 12 or 13 amino acids each that bind to cell surface heparin-like receptors (A5G27 and A5G33) were found to promote strong keratinocyte attachment, whereas the one that binds to integrin (A99) was inactive. Recombinant human collagen III (rhCol-III) gel was used as a delivery vehicle for cultured autologous skin cells (keratinocytes only or keratinocyte-fibroblast mixtures) [76]. Its effect on the healing of full-thickness wounds in a porcine wound-healing model was examined. Two Landrace pigs were used for the study. Fourteen deep dermal wounds were created on the back of each pig with an 8-mm biopsy punch. The scaffold enhanced early granulation tissue formation. Interestingly, fibroblast-containing gel was effectively removed from the wound, whereas gels without cells or with keratinocytes only remained intact.

5. Gene delivery systems in wound healing

Gene delivery is an emerging technology in the field of tissue repair and is being used to promote wound healing. Gene delivery is targeted to develop sustained release, to reduce side effects, and to enable both spatial and temporal control of gene silencing afterward. For example, chemical modifications were used to stabilize and reduce nonspecific effects of

siRNA molecules using effective delivery [77]. The controlled delivery of nucleic acids (DNA and RNA) to selected tissues remains an inefficient process are affected by low transfection efficacy, poor scalability because of varying efficiency with cell type and location, and questionable safety as a result of toxicity issues arising from the typical materials (e.g., viral vectors) and procedures employed. Biocompatible materials, in the formats of micro/nanoparticles, scaffolds, hydrogels and electrospun fibers, made from cationic polymers and lipids, have been used as nonviral vectors, which has attracted much attention recently.

5.1. Viral vectors in gene delivery

The TGF β family plays a critical regulatory role in repair and coordination of remodeling after cutaneous wounding. TGF β 3 has been implicated in an antagonistic role regulating overt wound closure and promoting ordered dermal remodeling. A mutant form of TGF β 3

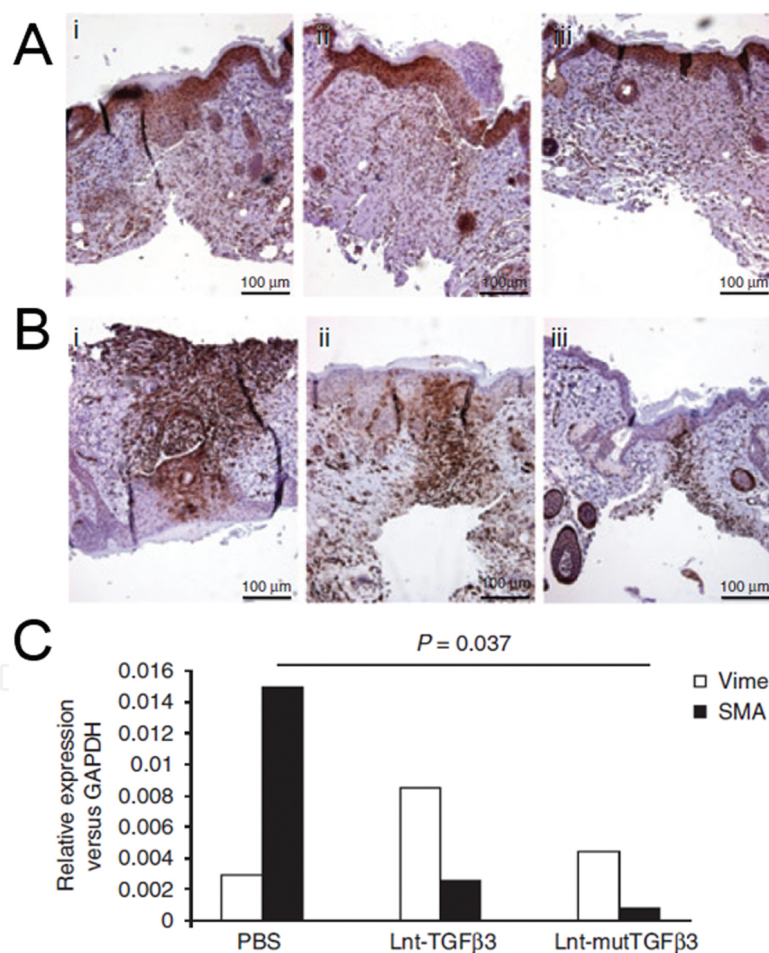


Figure 2. Transgenic overexpression of TGF β 3 decreases fibroblast to myofibroblast differentiation at the site of cutaneous wounding *in vivo*. (A) and (B) wound sections were stained immunohistochemically for fibroblast (a: vimentin) and myofibroblast (b: SMA) markers after treatment with [a and b(i)] PBS, [a and b(ii)] Lnt-TGF β 3, or [a and b(iii)] Lnt-mutTGF β 3. (C) Real-time reverse transcription-PCR showed that both TGF β 3 application groups and the PBS control ($n = 4$) as well as a significant decrease between the Lnt-mutTGF β 3 and Lnt-TGF β 3 treatment groups. PBS, phosphate-buffered saline; SMA, smooth muscle actin; TGF, transforming growth factor.

(mutTGF β 3) was generated by ablating its binding site for the latency-associated TGF β -binding protein (LTBP-1) [78]. A localized intradermal transduction using a lentiviral vector expressing the mutTGF β 3 in a mouse skin wounding model was demonstrated to reduce reepithelialization density and fibroblast/myofibroblast trans-differentiation within the wound area. Both of which reduced scar tissue formation (**Figure 2**). Using a noninvasive imaging system, the kinetics of luciferase gene expression was studied when delivered in an adenoviral vector (replication-deficient adenovirus, Ad5). A peak of gene expression occurred at 7 days after delivery [79]. The esophageal cancer-related gene-4 (Ecrg4) delivering a viral-mediated gene was evaluated in a cutaneous wound healing model [80]. Both Ecrg4 mRNA and its protein product were localized to the epidermis, dermis, and hair follicles of healthy mouse skin.

5.2. Nonviral vectors in gene delivery

Gene delivery using adenoviral vectors in tissue regeneration is hindered by a short duration of transgene expression. A fibrin scaffold was used to enhance delivery of the adenovirus to a wound site, precluding the need for high repeated doses [81]. An anti-fibrotic interfering RNA (RNAi) delivery system using exogenous microRNA (miR)-29B was proposed to modulate ECM remodeling following cutaneous injury. A collagen scaffold was used as the carrier of (miR)-29B. The mRNA expressions of collagen type I and collagen type III were reduced up to 2 weeks after fibroblasts culture. *In vivo* evaluation in full-thickness wounds treated with miR-29B delivery revealed that collagen type III/I ratio and matrix metalloproteinase (MMP)-8 to TIMP-1 ratio were improved [82]. Porous (100 and 60 μ m) and nonporous (n-pore) hyaluronic acid-MMP hydrogels with encapsulated reporter (pGFPluc) or proangiogenic (pVEGF) plasmids are used as a scaffold-mediated gene delivery [83]. Alginate-DNA gels were used to treat diabetic wounds, which provided sustained release of bioactive factors, such as neuropeptides and VEGF [13]. Silver nanoparticles (AgNPs) can be further augmented for gene delivery applications. The biofunctionalized stable AgNPs with good DNA-binding ability for efficient transfection and minimal toxicity were developed [84]. Polyethylene glycol (PEG)-stabilized chitosan-g-polyacrylamide was used to modify AgNPs. To enhance the efficiency of gene transfection, the Arg-Gly-Asp-Ser (RGDS) peptide was immobilized on the surface of AgNPs. The transfection efficiency of AgNPs increased significantly after immobilization of the RGDS peptide reaching up to $42 \pm 4\%$ and $30 \pm 3\%$ in HeLa and A549 cells, respectively. The transfection efficiency was significantly higher than $34 \pm 3\%$ and $23 \pm 2\%$, respectively, with the use of polyethylenimine (PEI, 25 kDa).

For treating diabetic patients with a threat of limb amputations, genes of various growth factors have been proposed in delivery systems. A simple nonviral gene delivery using minicircle plasmid DNA encoding VEGF was combined with an arginine-grafted cationic dendrimer PAM-RG4 [85]. Mouse ASCs were transfected with DNA plasmid encoding VEGF or green fluorescent protein (GFP) using biodegradable poly (β -amino) esters (PBAE). Cells transfected with LipofectamineTM 2000, a commercially available transfection reagent, were included as controls. ASCs transfected using PBAEs showed an enhanced transfection efficiency and 12–15-folds higher VEGF production compared with the controls ($*P < 0.05$) [86]. Keratinocyte

growth factor-1 (KGF-1) DNA was delivered using NTC8385-VA1 plasmid, a novel minimalized, antibiotic-free DNA expression vector [87].

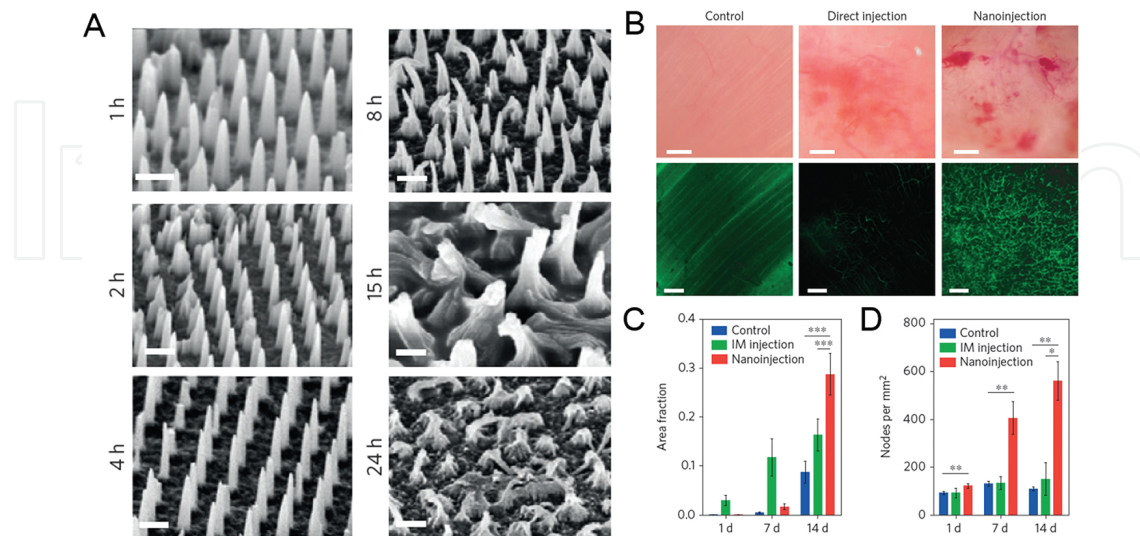


Figure 3. (A) Time course of nanoneedles incubated in cell-culture medium at 37°C. Scale bar = 2 μ m. (B) Nanoneedles mediate neovascularization in wound healing. (C) The number of nodes in the vasculature per millimeter square. (D) Within each field of view acquired for untreated control, intramuscular injection (IM), and nanoinjection. $P < 0.05$, $P < 0.01$, $P < 0.001$.

DNA-incorporated electrospun nanofibrous matrix was fabricated to control the release of DNA in response to high concentration of MMPs (matrix metalloproteinases) such as diabetic ulcers [88]. High efficiency and minimal toxicity *in vitro* have been demonstrated that can be used for an intracellular delivery of nucleic acids by using nanoneedles [89]. Biodegradable nanoneedles were fabricated by metal-assisted chemical etching of silicon. These nanoneedles mediated the *in situ* delivery of an angiogenic gene, VEGF165, and triggered the patterned formation of new blood vessels. The nanoneedles were designed for extremely localized delivery to a few superficial layers of cells (two-dimensional patterning). This gene delivery can access the cytosol to co-deliver DNA and siRNA with an efficiency greater than 90%. *In vivo* studies show that the nanoneedles transfected the VEGF165 gene, improved wound healing and scar-tissue remodeling, and induced sustained neovascularization and a localized sixfold increase in blood perfusion in the target region of the muscle (**Figure 3**). This confined intracellular delivery has the potential to target specific exposed areas within a tissue, further reduce the invasiveness of the injection, and limit the impact on the overall structure of the tissue.

6. Regulatory considerations

The major concerns of commercialization of drug/protein/cell/gene delivery wound dressings are the complicated registration process, specifically regulatory approval, protocol consideration, and clinical trial process. Among all the parameters of delivery wound dressings, the

type and source of the materials (e.g., human and animal origin) are critical to the regulatory approval process. A product composed of two or more regulated components, that is, drug/device, biologic/device, drug/biologic, or drug/device/biologic, that are physically, chemically, or otherwise combined or mixed and produced as a single entity is defined as a combination product [90]. The FDA (Food and Drug Administration, United States) regulation of a combination product (e.g., delivery system for wound healing) is mainly determined by the component with the primary mode of action. According to the classification of the product, the clinical trials (for premarket approval, PMA) must provide valid scientific evidence of safety and efficacy to support the indicated use of the wound healing delivery systems. Generally, preclinical studies contain toxicity studies and animal model evaluations. Delivery systems of drugs, bioactive proteins, cells, and genes in wound healing and nanomedicine should test their biocompatibility according to ISO 10993, including dermal irritation, dermal sensitization, cytotoxicity, acute systemic toxicity, hemocompatibility/hemolysis, pyrogenicity, mutagenicity studies, subchronic toxicity, chronic toxicity, and immunogenic potential [91]. Good clinical practices (GCPs) are the standards for designing, conducting, recording, and reporting clinical trials required for Class III medical devices.

For example, autologous stem cells are under clinical trial and are effective in ulcer healing and angiogenesis. However, translating delivery of stem cell application in *in vitro* and *in vivo* experiments from animal models to human clinical trials is still in its infancy. Preclinical studies suggest that cell delivery systems represent an effective and safe therapeutic strategy in the treatment of nonhealing wounds. More clinical studies on human subjects, including better data management of the patients and long-term follow-up of the patients' conditions, are necessary. Improved stem cell delivery vehicles in large-scale human clinical trials may be promising for diabetics with foot ulcers. There are no serious complications or side effects, but its therapeutic mechanisms, effects, and standardization still require further research [92]. While delivery system-based products offer increasingly important strategies for managing complex wounds, potential drawbacks include the risks of infectious agent transfer and immunological rejection. The manufacturing process, transport, and storage of delivery systems in wound healing are major cost implications; thus, their current clinical use remains limited [93]. Many current clinical trials are placing a high emphasis on addressing safety issues in all stem cell therapies, including stem cell delivery in wound healing [94]. The serious adverse effects of stem cell delivery are mainly immune response and tumorigenic potential. Delivery systems used in cell therapy encompass four main approaches, which are systemic administration, injection, topical, and local deliveries. Localized delivery of cells in wound healing is an optimal delivery approach for wound treatments [95]. Nonimmunogenic, nontoxic, biodegradable, and biocompatible biomaterials have been developed as carriers of stem cells that can protect cells and improve wound healing. However, clinical use of stem cells, for example, allogeneic EPCs, is currently inhibited by the risk of immunogenicity and tumorigenicity. To modulate the immune response, mesenchymal stromal cells or umbilical cord blood is already used in clinical trials, but definitive results are still pending. MSCs are known to be hypoimmunogenic [96]. Current challenges are standardized and quality-controlled cell therapy, the differentiation of MSCs to unwanted tissue, and potential tumorigenicity [94]. MSCs have been applied clinically for the treatment of diabetic wounds. Long

in vitro expansion time and multiple handling procedures are barriers for its clinical application and increase the chances of infection [97]. Autologous induced pluripotent stem cells are nonimmunogenic and can be a promising cell source used in wound healing [98]. By comparison, clinical use of allogeneic cells is more complex and requires additional regulatory, legal, and safety hurdles to be overcome [99]. All things considered, the future prospects for the utilization of both autologous and allogeneic cells in cell delivery systems are bright. In the United States, there are three regulatory processes for the registration of wound healing delivery systems [100]. Only wound dressing with lower complexity and risk that is substantially equivalent to a marketed “predicate” device may be cleared through the 510(k) premarket notification process. In another words, those types of wound dressings are classified as Class I medical device. Clinical data are typically not needed for 510(k) clearance of Class II medical devices. Higher-risk Class III medical devices typically require premarket approval (PMA). In summary, the regulatory processes are depending on multiple factors including the device's classification, the availability of a substantially equivalent predicate, and the level of risk. Before commercialization, investigational devices maybe clinically investigated within the USA through the investigational device exemption (IDE) process, which is a request to conduct clinical research on an investigational device with “significant” risk in the **United States**.

User fees are required with the submissions of 510(k) premarket notifications and PMA application in the **United States**. Recently, Health Canada released a consultation document that discusses the cost recovery (user fee) framework which shows the basis for accountability at Health Canada for the review process [101]. Essentially, the fees “guarantee” a certain level of service from Health Canada—for instance, specifying the target number of days in which Health Canada will process different types of applications. If the targets are not met, that is, if “performance” does not meet the established standard, the entity being charged the user fee will have their future fees reduced by a corresponding amount. Providing a framework for registration approval globally of delivery wound dressings would translate those delivery systems studied from the laboratory investigation stage to clinical use, which will benefit patients’ quality of life.

7. Conclusions

In the past few decades, many wound dressings and skin substitutes have been developed to treat skin loss and wounds. Delivery systems have been proven to improve wound healing and skin tissue regeneration. Polymeric microspheres and nanospheres, nanoparticles, nanofibrous structures, hydrogels, and scaffolds have been developed to deliver drugs to wound sites, overcoming the challenges caused by antibiotic-resistant microbial infections. Controlled release of drug delivery systems has been of increasing interest, as well as the applications of nanotechnology and biomaterial scaffolds. Growth factor and peptide delivery systems applied in skin wound healing help in the regeneration of tissue, reduction of scarring, and reconstruction of blood capillaries (neovascularization). Keratinocytes, fibroblasts, endothelial cells, mesenchymal stem cells, adipose-derived stem cells, and endothelial progenitor cells studied in delivery systems have great promise in chronic wounds and diabetic

ulcers. Gene therapies now in clinical trials and the discovery of biodegradable polymers, fibrin meshes, and human collagen serving as potential delivery systems may soon be available to clinical wound management. However, regeneration of peripheral nerves is seldom reported. Looking toward the future, these delivery wound healing products may be able to achieve the replacement and regeneration of more normal skin; to gain localized delivery to wound site; to heal severe burns, chronic and complex wounds; to control the release of drugs, growth factors, and cells; and to silence genes.

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